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Coming up for air: HIF-1 and mitochondrial oxygen consumption

Hypoxic cells induce glycolytic enzymes; this HIF-1-mediated metabolic adaptation increases glucose flux to pyruvate and produces glycolytic ATP. Two papers in this issue of *Cell Metabolism* (Kim et al., 2006; Papandreou et al., 2006) demonstrate that HIF-1 also influences mitochondrial function, suppressing both the TCA cycle and respiration by inducing pyruvate dehydrogenase kinase 1 (PDK1). PDK1 regulation in hypoxic cells promotes cell survival.

Oxygen deprivation (hypoxia) occurs in tissues when O_2 supply via the cardiovascular system fails to meet the demand of O_2-consuming cells. Hypoxia occurs naturally in physiological settings (e.g., embryonic development and exercising muscle), as well as in pathophysiological conditions (e.g., myocardial infarction, inflammation, and solid tumor formation). For over a century, it has been appreciated that O_2-deprived cells exhibit increased conversion of glucose to lactate (the "Pasteur effect"). Activation of the Pasteur effect during hypoxia in mammalian cells is facilitated by HIF-1, which mediates the upregulation of glycolytic enzymes that support an increase in glycolytic ATP production as mitochondria become starved for O_2, the substrate for oxidative phosphorylation (Seagroves et al., 2001). Thus, mitochondrial respiration passively decreases due to O_2 depletion in hypoxic tissues. However, reports by Kim et al. (2006) and Papandreou et al. (2006) in this issue of *Cell Metabolism* demonstrate that this critical metabolic adaptation is more complex and includes an active suppression of mitochondrial pyruvate catabolism and O_2 consumption by HIF-1.

Mitochondrial oxidative phosphorylation is regulated by multiple mechanisms, including substrate availability. Major substrates include O_2 (the terminal electron acceptor) and pyruvate (the primary carbon source). Pyruvate, as the end product of glycolysis, is converted to acetyl-CoA by the pyruvate dehydrogenase enzymatic complex and enters the tricarboxylic acid (TCA) cycle. Pyruvate conversion into acetyl-CoA is irreversible; this therefore represents an important regulatory point in cellular energy metabolism. Pyruvate dehydrogenase kinase (PDK) inhibits pyruvate dehydrogenase activity by phosphorylating its E1 subunit (Sugden and Holness, 2003). In the manuscripts by Kim et al. (2006) and Papandreou et al. (2006), the authors find that PDK1 is a HIF-1 target gene that actively regulates mitochondrial respiration by limiting pyruvate entry into the TCA cycle. By excluding pyruvate from mitochondrial metabolism, hypoxic cells accumulate pyruvate, which is then converted into lactate via lactate dehydrogenase (LDH), another HIF-1-regulated enzyme. Lactate in turn is released into the extracellular space, regenerating NAD^+ for continued glycolysis by O_2-starved cells (see Figure 1). This HIF-1-dependent block to mitochondrial O_2 consumption promotes cell survival, especially when O_2 deprivation is severe and prolonged.

Papandreou et al. demonstrate that hypoxic regulation of PDK has important implications for antitumor therapies. Recent interest has focused on cytotoxins that target hypoxic cells in tumor microenvironments, such as the drug tirapazamine (TPZ). Because intracellular O_2 concentrations are decreased by mitochondrial O_2 consumption, HIF-1 could protect tumor cells from TPZ-mediated cell death by maintaining intracellular O_2 levels. Indeed, Papandreou et al. show that HIF-1-deficient cells grown at 2% O_2 exhibit increased sensitivity to TPZ relative to wild-type cells, presumably due to higher rates of mitochondrial O_2 consumption. HIF-1 inhibition in hypoxic tumor cells should have multiple therapeutic benefits, but the use of HIF-1 inhibitors in conjunction with other treatments has to be carefully evaluated for the most effective combination and sequence of drug delivery. One result of HIF-1 inhibition would be a relative decrease in intracellular O_2 levels, making hypoxic cytotoxins such as TPZ more potent antitumor agents. Because PDK expression has been detected in multiple human tumor samples and appears to be induced by hypoxia (Koukourakis et al., 2005), small molecule inhibitors of HIF-1 combined with TPZ represent an attractive therapeutic approach for future clinical studies.

Hypoxic regulation of PDK1 has other important implications for cell survival during O_2 depletion. Because the TCA cycle is coupled to electron transport, Kim et al. suggest that induction of the pyruvate dehydrogenase complex by PDK1 attenuates not only mitochondrial respiration but also the production of mitochondrial reactive oxygen species (ROS) in hypoxic cells. ROS are a byproduct of electron transfer to O_2, and cells cultured at 1 to 5% O_2 generate increased

mitochondrial ROS relative to those cultured at 21% O_2 (Chandel et al., 1998; Guzy et al., 2005). In fact, hypoxia-induced mitochondrial ROS have also been shown to be necessary for the stabilization of HIF-1 in hypoxic cells (Brunelle et al., 2005; Guzy et al., 2005; Mansfield et al., 2005). However, the persistence of ROS could ultimately be lethal to tissues during chronic O_2 deprivation, and PDK1 induction by HIF-1 should promote cell viability during long-term hypoxia. Kim et al. present evidence that HIF-1-deficient cells exhibit increased apoptosis after 72 hr of culture at 0.5% O_2 compared to wild-type cells and that cell survival is rescued by enforced expression of exogenous PDK1. Furthermore, PDK1 reduces ROS production by the HIF-1 null cells. These findings support a novel prosurvival dimension of cellular hypoxic adaptation where PDK1 inhibits the TCA cycle, mitochondrial respiration, and chronic ROS production.

The HIF-1-mediated block to mitochondrial O_2 consumption via PDK1 regulation also has implications for O_2-sensing pathways by hypoxic cells. One school of thought suggests that perturbing mitochondrial O_2 consumption increases intracellular O_2 concentrations and suppresses HIF-1 induction by promoting the activity of HIF prolyl hydroxylases, the O_2-dependent enzymes that regulate HIF-1 stability (Hagen et al., 2003; Doege et al., 2005). This model suggests that mitochondria function as "O_2 sinks." Although Papandreou et al. demonstrate that increased mitochondrial respiration due to PDK1 depletion results in decreased intracellular O_2 levels (based on pimonidazole staining), these changes failed to reduce HIF-1 levels in hypoxic cells. Another model for hypoxic activation of HIF-1 describes a critical role for mitochondrial ROS in prolyl hydroxylase inhibition and HIF-1 stabilization in O_2-starved cells (Brunelle et al., 2005; Guzy et al., 2005; Mansfield et al., 2005) (see Figure 1). The mitochondrial "O_2 sink" hypothesis can account for some observations in the literature but fails to explain the inhibition of HIF-1 stabilization by ROS scavengers (Chandel et al., 1998; Brunelle et al., 2005; Guzy et al., 2005; Sanjuán-Pla et al., 2005). While the relationship between HIF-1 stability, mitochondrial metabolism, ROS, and intracellular O_2 redistribution will continue to be debated for some time, these most recent findings shed new light on findings by Louis Pasteur over a century ago.

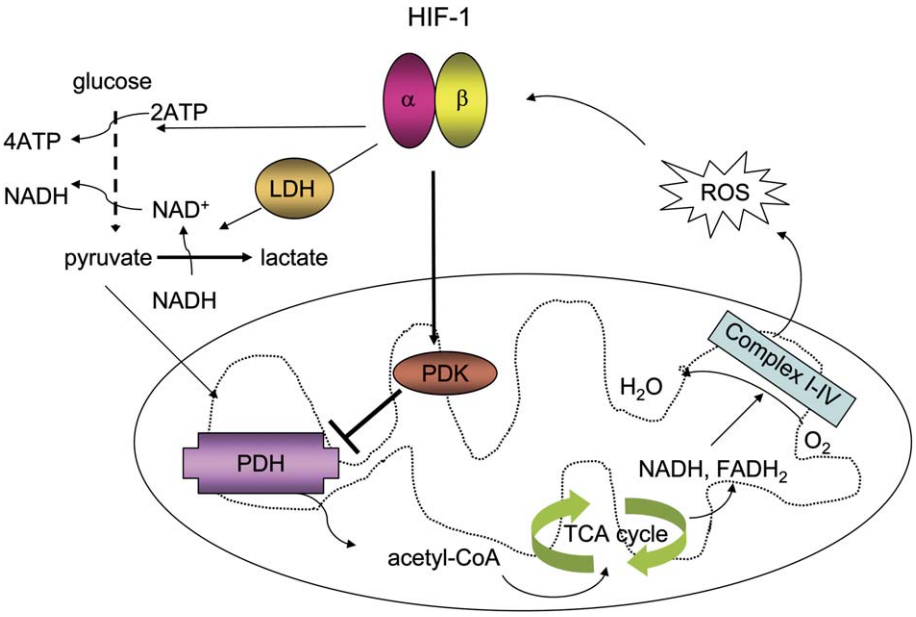

Figure 1. Multiple hypoxia-induced cellular metabolic changes are regulated by HIF-1

By stimulating the expression of glucose transporters and glycolytic enzymes, HIF-1 promotes glycolysis to generate increased levels of pyruvate. In addition, HIF-1 promotes pyruvate reduction to lactate by activating lactate dehydrogenase (LDH). Pyruvate reduction to lactate regenerates NAD^+, which permits continued glycolysis and ATP production by hypoxic cells. Furthermore, HIF-1 induces pyruvate dehydrogenase kinase 1 (PDK1), which inhibits pyruvate dehydrogenase and blocks conversion of pyruvate to acetyl CoA, resulting in decreased flux through the tricarboxylic acid (TCA) cycle. Decreased TCA cycle activity results in attenuation of oxidative phosphorylation and excessive mitochondrial reactive oxygen species (ROS) production. Because hypoxic cells already exhibit increased ROS, which have been shown to promote HIF-1 accumulation, the induction of PDK1 prevents the persistence of potentially harmful ROS levels.

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